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Effects of 1,2,4-Trichlorobenzene on Estuarine Macrobenthic Communities Exposed via Water and Sediment

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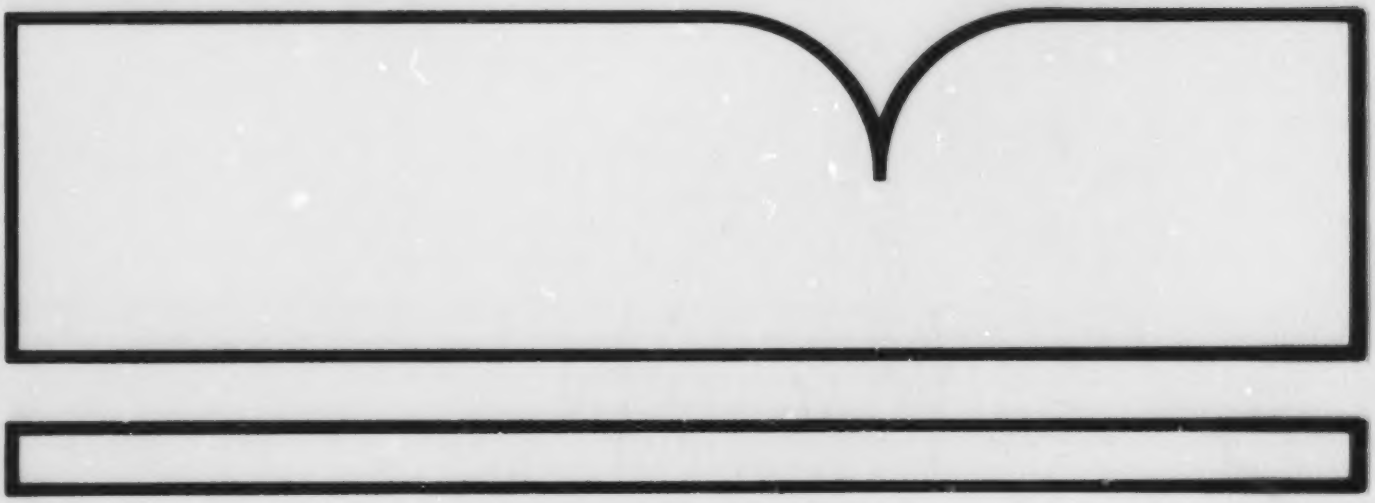

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16. ABSTRACT

Macrobenthic animal communities that colonized sand-filled aquaria were exposed to 1,2,4-trichlorobenzene (TCB), a recent replacement for polychlorinated biphenyls in the electrical industry. In one test, communities established by planktonic larvae entrained in continuously supplied unfiltered seawater for 50 days were exposed to waterborne TCB for 6 days; in the second, the toxicant was added to the sediment before 8 weeks of colonization. Concentrations that affected community structure were usually two orders of magnitude lower for waterborne TCB than for sediment-bound TCB, but the same types of organisms were affected by each route of exposure. The lowest TCB concentrations (measured) that affected average numbers of individuals exposed via the water were 0.04 mg/liter for mollusks, 0.4 mg/liter for arthropods, and 4 mg/liter for annelids. Average number of species was significantly lower than the control at 4 mg/liter. TCB persisted in sediments, but some leached into water throughout the 8-week exposure via sediment.

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ECOTOXICOLOGY AND ENVIRONMENTAL SAFETY 10, 351–360 (1985)

Effects of 1,2,4-Trichlorobenzene on Estuarine Macrobenthic Communities Exposed via Water and Sediment

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Received April 16, 1985

Macrobenthic animal communities that colonized sand-filled aquaria were exposed to 1,2,4-trichlorobenzene (TCB), a recent replacement for polycholorinated biphenyls in the electrical industry. In one test, communities established by planktonic larvae entrained in continuously supplied unfiltered seawater for 50 days were exposed to waterborne TCB for 6 days; in the second test, the toxicant was added to the sediment before 8 weeks of colonization. Concentrations that affected community structure were usually two orders of magnitude lower for waterborne TCB than for sediment-bound TCB, but the same types of organisms were affected by each route of exposure. The lowest TCB concentrations (measured) that affected average numbers of individuals exposed via the water were 0.04 mg/liter for mollusks, 0.4 mg/liter for arthropods, and 4 mg/liter for annelids. Average number of species was significantly lower than the control at 4 mg/liter. For TCB exposures via the sediment, the lowest concentrations (nominal) that affected average numbers of individuals were 100 μg/g for mollusks and echinoderms, and 1000 μg/g for arthropods and annelids. Average number of species in experimental aquaria was significantly lower than the control at >100 μg/g. TCB persisted in sediments, but some leached into water throughout the 8-week exposure via sediment. © 1985 Academic Press, Inc.

INTRODUCTION

1,2,4-Trichlorobenzene (TCB), $C_6H_3Cl_3$, has many industrial uses and its production is second by weight among chlorinated benzenes in the United States (EPA, 1980). It is primarily a dye carrier in the textile industry, but also serves as a herbicide intermediate, heat transfer medium, soil insecticide against termites, degreaser, lubricant, and dielectric fluid in transformers.

Present low concentrations of TCB reported in the aquatic environment are expected to increase with increased production. Concentrations up to 500 μg/liter were found in Tennessee and North Carolina creeks after industrial discharges and 0.007 μg/liter occurred in the Los Angeles River from surface runoff (EPA, 1980). However, TCB is now being used with di(2-ethylhexyl)phthalate (DEHP) as a replacement for banned polychlorinated biphenyls (PCB) in dielectric fluids. If TCB becomes a major PCB-replacement compound in the electrical industry, it is anticipated that production in the United States, about 1000 metric tons a year, will double and environmental concentrations will increase (Addison, 1983; Smith *et al.*, 1980). In addition, accidental spills of dielectric fluids into the environment have occurred, and probably will continue to occur (Addison, 1983).

As Addison (1983) pointed out, much less is known about the biological effects of PCB replacements than about the PCBs themselves. In addition, Saperstein and Faeder (1982) stated that the TCB/DEHP mixture appears to have a greater potential for significant environmental impact than other PCB-substitute fluids currently available. Various investigators have shown that TCB is toxic to some aquatic organisms. Acute

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toxicity (48 or 96 hr LC_{50}/EC_{50}) of TCB to four species of fishes ranged from 1.5 to 21.4 mg/liter; to invertebrates, 50.2 mg/liter for *Daphnia magna* and 0.45 mg/liter for *Mysidopsis bahia* (EPA, 1980). Chronic values for fishes in embryo–larval tests were 0.286 mg/liter for *Pimephales promelas* and 0.222 mg/liter for *Cyprinodon variegatus* (EPA, 1980). Fish and invertebrates can also accumulate this chemical from water. Steady-state bioconcentration factors were 183× for *Lepomis macrochirus* and 142× for *D. magna* (Macek *et al.*, 1979).

A PCB (Aroclor 1254) and a phthalate (di-*n*-butyl phthalate) have been shown to alter the composition of estuarine benthic communities (Hansen, 1974; Tagatz *et al.*, 1983), but the effect of TCB on such communities is not known. The present study reports the responses of estuarine benthic communities to TCB exposure via water and sediment. Waterborne toxicants ultimately may accumulate in sediments which then act as reservoirs to recycle toxicants back into the aquatic environment. The high octanol–water partition coefficient of 4.26 indicates that sorption of TCB by sediment may be substantial at concentrations anticipated in environmental water (EPA, 1979).

MATERIALS AND METHODS

Water exposure. Effects of a 6-day exposure (November 15 to 21, 1983) of TCB in water on macrobenthic animals that colonized sand-filled aquaria for 50 days (September 26 to November 15, 1983) were determined by comparing community structures in contaminated and uncontaminated aquaria. Aquaria were colonized by settling of planktonic larvae entrained in continuously flowing unfiltered seawater from Santa Rosa Sound, Florida. We used four acrylic plastic apparatuses (Fig. 1), each consisting

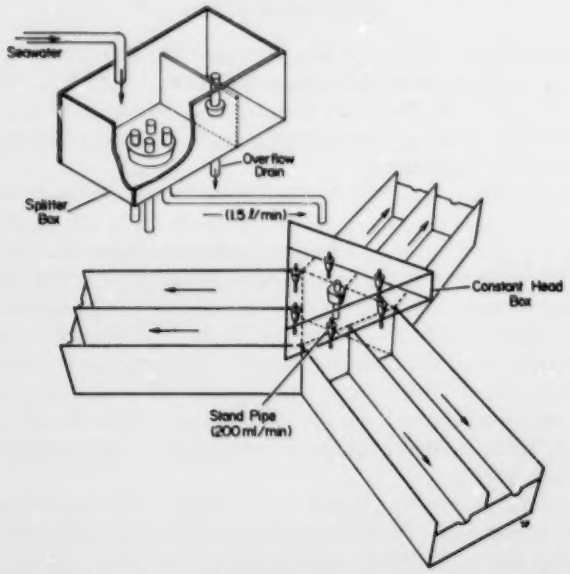

FIG. 1. One of four identical apparatuses of six aquaria used to test the effects of TCB in water or sediment on macrobenthic communities. The splitter box supplied seawater to the four apparatuses.

of a central constant-head box atop six aquaria (40 cm long, 10 cm wide, and 12 cm high). Aquaria were filled to a depth of 5.5 cm with clean silica sand collected more than 6 months previously from Santa Rosa Sound. Ninety-eight percent of particles were 0.26 to 1.00 mm. Water level in aquaria was maintained at 3 cm above the sand.

Water flowed to a splitter box from which four adjacent glass tubes supplied it at 1.5 liter/min to the four constant-head boxes that routed water to the aquaria (Fig. 1). Flow to each aquarium was maintained at 200 ml/min by adjusting the height of a glass standpipe (2.2 mm i.d.) in the constant-head box. Water flowed from each aquarium through a notched end opening. Salinity and temperature of seawater entering the laboratory, recorded as daily minimum and maximum, averaged 28.0‰ (21.0 to 32.5‰) and 22.0°C (17.0 to 25.5°C) during colonization before exposure. Photoperiod was 12-hr light:12-hr dark; light energy at test surface was 6.4 $\mu E/sec/m^2$.

One apparatus served as a control and three received nominal concentrations of 0.05, 0.5, and 5 mg TCB/liter. TCB (99⁺% pure), dissolved in acetone was metered by pump through polyethylene tubing and injected through a side opening in the glass tubing that carried seawater to each treated apparatus. An equal amount of carrier solvent (0.26 ml acetone/liter dilution water) was metered into the water that entered the control aquaria. Water samples, taken on Days 2, 3, and 6 from each head box during the 6-day exposure, were analyzed for TCB content. A peristaltic tubing pump was used to pass 1-liter samples through XAD-4 resin traps simultaneously from the four head boxes over a period of 1 hr. Data are presented as average measured concentrations. Salinity during exposure averaged 30.0‰ (28.5 to 32.0‰); temperature, 18.5°C (17.0 to 20.0°C).

The communities were harvested after exposure to TCB. Animals retained by a 1-mm mesh sieve were preserved, counted, and identified.

Sediment exposure. Responses of macrobenthic animals exposed for 8 weeks (April 23 to June 18, 1984) to sediment contaminated with TCB were determined by comparing communities that developed in aquaria containing contaminated and uncontaminated sand. Aquaria were filled to a depth of 5.5 cm with the same stock of sand from Santa Rosa Sound as used in the water exposures. Aquaria, water flows, photoperiod, and method of colonization were as described earlier (Fig. 1). Salinity and temperature of seawater entering the laboratory, recorded as daily minimum and maximum, averaged 26.0‰ (15.5 to 32.5‰) and 24°C (18 to 28°C) during colonization.

Aquaria were filled with clean sand (the control) or sand mixed with varying amounts of TCB. Appropriate amounts of TCB in acetone and sand were mixed by hand for each treated apparatus so that each aquarium received TCB (0.024, 0.24, or 2.42 ml) in 50 ml acetone and 2200 cc wet sand. An equal amount of acetone (50 ml) was blended into control aquaria. Data are presented as nominal concentrations of TCB (0, 10, 100, and 1000 $\mu g/g$) or as nominal concentrations designated as low (10 $\mu g/g$), medium (100 $\mu g/g$), and high (1000 $\mu g/g$).

Samples of overlying water were taken after Days 1 and 7, then weekly from a control aquarium and from an aquarium at each TCB concentration and analyzed for TCB that leached from the sand. As before, a peristaltic pump was used to obtain 1-liter samples through resin traps over the period of 1 hr.

Core samples of sand were taken from each concentration at the beginning of the test, after Days 1 and 7, then weekly with a modified 50-cc syringe (opening enlarged

to full diameter). These were analyzed to determine reduction of TCB in sand during the 8-week study. To avoid disturbing the integrity of the benthic communities, sand samples were taken from an additional apparatus (three aquaria for TCB concentrations and three for sand) connected by a fifth tube from the splitter box shown in Fig. 1. We prepared sufficient amounts of the sand/TCB mixtures to fill these additional aquaria to 5.5 cm depth. They were maintained under the same experimental conditions, but used only to supply sand samples.

After 8 weeks of colonization, benthic communities were harvested. Animals retained by a 1-mm mesh sieve were preserved, counted, and identified.

Statistical and chemical analyses. Effects of TCB exposure via the water and sediment on numbers of individuals and species were determined by a one-way analysis of variance performed on all phyla combined and by individual phylum when numbers were sufficient to justify such an analysis. The analyses were performed after applying a square-root transformation (a variance-stabilizing transformation) to the observed counts. Differences were considered significant at the 0.05 level. Duncan's multiple comparison procedure (Winer, 1971) was used to determine which exposure groups differed when an overall significant effect was detected.

XAD-4 resin traps used to remove TCB from 1-liter water samples were washed with 5 ml deionized water and excess water removed by vacuum before test compound was eluted with 20 ml of acetone. The acetone extract was concentrated to 2 ml with a gentle stream of nitrogen at 35°C and either diluted with petroleum ether to an appropriate volume or injected directly into the gas chromatograph for quantitative analyses. Gas chromatographic conditions were injector temperature, 200°C; column temperature, 120°C; electron-capture detector temperature, 300°C. A 122 cm × 2 mm (i.d.) glass column packed with 2% SP2100 on 100/120 mesh Supelcoport was used for separation.

Sediment samples were extracted thrice with 20 ml acetone and analyzed according to the procedure for seawater. Cleanup was not necessary for these samples prior to gas chromatographic analyses.

Samples of seawater and sediment were fortified for quality assurance of chemical analyses. Average percentage recoveries were 87 ± 6.7 (n = 9) for seawater fortified at 1.0 μg/liter and 94 ± 6.9 (n = 10) for sediment fortified at 10 μg/g.

RESULTS

Water Exposure

TCB water concentrations, measured during the 6-day exposure, were nondetectable (control), 0.044 mg/liter ± 0.004, 0.36 mg/liter ± 0.07, and 3.9 mg/liter ± 1.8. These averages were rounded off to 0.04, 0.4, and 4 mg/liter in the presentation of data.

A total of 1628 animals representing 27 species of seven phyla was collected from control aquaria and aquaria exposed to TCB via water (Table 1). Arthropods (primarily *Corophium acherusicum*), mollusks (primarily *Laevicardium mortoni*), and annelids (primarily *Armandia maculata*) were the most abundant phyla.

Community structure was significantly altered by waterborne TCB (Table 2). For all phyla combined, there were significantly lower average numbers of individuals at all concentrations than in the control. Average number of species for combined phyla was lower only at the highest concentration of TCB, 4 mg/liter. The lowest TCB concentrations that affected numbers of individuals were 0.04 mg/liter for mollusks,

TABLE 1

ANIMALS COLLECTED FROM CONTROL AQUARIA AND FROM AQUARIA THAT RECEIVED TCB VIA WATER FOR 6 DAYS[a]

Taxon	Control	TCB (mg/liter), measured			Total animals
		0.04	0.4	4	
Arthropoda					
Corophium acherusicum	339	271	227	0	837
Balanus amphitrite	8	11	17	4	40
Neopanope texana	0	1	3	0	4
Heterocrypta granulata	0	1	0	0	1
Total arthropods	347	284	247	4	882
Mollusca					
Laevicardium mortoni	136	79	81	0	296
Diastoma varium	13	8	10	2	33
Acteocina canaliculata	11	8	3	2	24
Musculus lateralis	4	1	3	3	11
Tellina sp.	1	3	0	0	4
Anadara notabilis	0	1	2	0	3
Mulinia lateralis	2	1	0	0	3
Mitrella lunata	0	1	0	1	2
Ancula evelinae	1	0	0	0	1
Crepidula maculosa	1	0	0	0	1
Crepidula plana	0	1	0	0	1
Total mollusks	169	103	99	8	379
Annelida					
Armandia maculata	81	69	85	2	237
Pomatoceros americanus	19	12	12	4	47
Hydroides dianthus	2	2	1	1	6
Pectinaria gouldii	2	0	1	0	3
Neanthes succinea	0	1	2	0	3
Polydora sp.	0	0	1	0	1
Unidentified Sabellidae	1	0	0	0	1
Total annelids	105	84	102	7	298
Echinodermata					
Leptosynapta inhaerens	10	7	5	3	25
Hemipholis elongata	1	0	2	0	3
Total echinoderms	11	7	7	3	28
Chordata					
Molgula manhattensis	9	3	6	5	23
Cnidaria					
Unidentified Actiniaria	7	3	5	2	17
Brachiopoda					
Glottidia pyramidata	0	0	0	1	1
All phyla					
Individuals	648	484	466	30	1628
Species	19	20	18	12	27

[a] Replicates were pooled.

TABLE 2

AVERAGE NUMBER OF ANIMALS AND AVERAGE NUMBER OF SPECIES IN CONTROL AQUARIA AND IN AQUARIA EXPOSED TO TCB VIA WATER

Taxon	Control	TCB (mg/liter), measured		
		0.04	0.4	4
Arthropoda	57.8 (5.5)[a]	47.3 (6.4)	41.2* (5.9)	0.7* (0.5)
Mollusca	28.2 (5.0)	17.2* (1.7)	16.5* (1.9)	1.3* (0.5)
Annelida	17.5 (2.0)	14.0 (2.1)	17.0 (1.7)	1.2* (0.8)
Echinodermata	1.8 (1.0)	1.2 (1.2)	1.2 (0.4)	0.5 (0.5)
Chordata	1.5 (0.7)	0.5 (0.3)	1.0 (0.6)	0.8 (0.4)
Cnidaria	1.2 (0.4)	0.5 (0.2)	0.8 (0.5)	0.3 (0.2)
Brachiopoda	0	0	0	0.2 (0.2)
All Phyla				
Individuals	108.0 (7.7)	80.7* (6.4)	77.7* (8.0)	5.0* (1.2)
Species	9.5 (0.8)	8.5 (1.0)	9.7 (0.2)	3.5* (0.6)

[a] Standard error in parentheses.
* Significantly different from control ($\alpha = 0.05$, analysis performed on square-root transformed counts).

0.4 mg/liter for arthropods, and 4 mg/liter for annelids. Average numbers of species of mollusks, arthropods, and annelids showed significant decreases from control numbers only at the highest exposure of TCB.

Distress occurred 2 hr after the beginning of TCB exposure among mollusks and annelids in aquaria that received 4 mg/liter, and among mollusks in aquaria that received 0.4 mg/liter: 1 to 5 *L. mortoni* per aquarium and the same numbers of *A. maculata*, both infaunal species, emerged fully from the sediment. Deaths occurred among *L. mortoni* (4 to 12 mm high) and *A. maculata* (10 to 15 mm long) during the next 22 hr of exposure in the high concentration. Numbers of deaths observed per replicate at 24 hr were 13, 3, 17, 4, 3, and 8 for the mollusk and 12, 15, 8, 14, 12 and 12 for the annelid. Mollusks in distress in the medium concentration at 2 hr burrowed into the sand by 24 hr, and no deaths were observed. Only a few dead amphipods (*C. acherusicum*) were noted in medium and high concentrations of TCB during the first 24 hr of exposure.

Sediment Exposure

Although TCB continually leached from sediment to water, it persisted in sediment throughout the test (Table 3). Measured concentrations of TCB in samples of sediment and water from aquaria containing sediment at nominal concentrations of 10, 100, and 1000 μg/g fluctuated with time, but after 8 weeks, values were <0.01, 6.1, and 519 μg/g, respectively, for sediment and 0.51, 12, and 74 μg/liter, respectively, for water.

A total of 937 animals representing 31 species of seven phyla was collected from control aquaria and from aquaria that contained TCB via sediment (Table 4). Arthropods (primarily *C. acherusicum*), annelids (primarily *Neanthes succinea*), and mollusks (primarily *Mulinia lateralis*) were the most adundant phyla.

TABLE 3

MEASURED CONCENTRATIONS OF TCB IN SEDIMENT (NOMINAL CONCENTRATIONS, 10, 100, AND 1000 μg/g) AND IN OVERLYING WATER

	μg/g sediment			μg/liter water		
Days elapsed	10	100	1000	10	100	1000
0	4.3	81	676	—	—	—
1	1.6	97	590	1.21	11	89
7	0.037	3.8	594	0.73	47	21
14	0.028	35	697	0.073	19	16
21	0.16	62	709	0.37	14	38
28	0.14	2.7	790	0.38	15	14
36	0.056	38	673	0.011	37	21
42	0.031	2.1	730	0.18	24	69
49	0.15	42	768	0.13	18	113
56	<0.01	6.1	519	0.51	12	74

Community structure was significantly affected by sediment-bound TCB (Table 5). For all phyla combined, a significant decrease from the control in the average numbers of individuals and species occurred at 100 and 1000 μg/g. The lowest TCB concentrations that affected numbers of individuals by phyla were 1000 μg/g for arthropods and annelids, and 100 μg/g for mollusks and echinoderms. Abundance of the dominant arthropod, *C. acherusicum*, also was less than that in the control at 100 μg/g and above. Mollusca was the only phylum for which average numbers of species in contaminated aquaria were significantly less than those in control aquaria in both medium and high concentrations. No distress was detected among exposed mollusks or annelids as was observed on the first day of the waterborne TCB test.

DISCUSSION

Persistence of TCB in sand was due in part to sorption by organic material that accumulated on the sediment. Organic content in sand samples (difference between dry and ashed weights) increased from 0 to 0.24% during the 8-week test. This value lies within the range of concentrations (0.18 to 5.64%) found in samples of natural sediment from four northwest Florida bays and sounds (Stoner, 1980; Stoner *et al.*, 1983; Sheridan and Livingston, 1983).

While mortality often could be determined empirically to document toxicant effect of waterborne TCB on established benthic communities, few bodies were observed in sediment-bound TCB studies where the toxicant was introduced before colonization. Larvae may have avoided settling or failed to develop in the contaminated sediments.

Communities exposed via water or sediment were similar and the same types of organisms were affected by TCB, regardless of method of exposure. The same three phyla were dominant and more than one-half of the species occurred in both studies. Also, in both instances, mollusks were the most sensitive. Abundance of the dominant arthropod, *C. acherusicum*, was less than that in the control at the two higher concentrations tested in each study. In each type of exposure, annelids were affected only by the highest concentration. The dominant echinoderm, *Leptosynapta inhaerens*,

TABLE 4

ANIMALS COLLECTED FROM CONTROL AQUARIA AND FROM AQUARIA THAT CONTAINED TCB VIA SEDIMENT[a]

Taxon	Control	TCB (μg/g), nominal			Total animals
		10	100	1000	
Arthropoda					
Corophium acherusicum	118	76	50	19	263
Balanus amphitrite	24	30	24	19	97
Neopanope texana	0	2	2	3	7
Total arthropods	142	108	76	41	367
Annelida					
Neanthes succinea	35	45	34	21	135
Armandia maculata	5	7	5	4	21
Hydroides dianthus	5	5	1	6	17
Pectinaria gouldii	7	6	0	0	13
Pomatoceros sp.	3	0	1	3	7
Prionospio heterobranchia	4	1	1	0	6
Capitellides jonesi	0	0	2	1	3
Polydora socialis	0	0	2	1	3
Capitella capitata	0	1	0	0	1
Haploscoloplos sp.	0	1	0	0	1
Piromis roberti	1	0	0	0	1
Unidentified Sabellidae	0	1	0	0	1
Total annelids	60	67	46	36	209
Mollusca					
Mulinia lateralis	39	45	12	0	96
Laevicardium mortoni	19	25	3	2	49
Musculus lateralis	8	10	6	0	24
Acteocina canaliculata	5	5	0	0	10
Anadara transversa	1	1	0	2	4
Tellina alternata	2	2	0	0	4
Ensis minor	0	2	0	0	2
Crepidula maculosa	1	0	0	0	1
Crepidula plana	0	1	0	0	1
Lima pellucida	0	1	0	0	1
Mitrella lunata	0	1	0	0	1
Total mollusks	75	93	21	4	193
Chordata					
Molgula manhattensis	9	41	10	35	95
Echinodermata					
Leptosynapta inhaerens	23	20	11	2	56
Unidentified Amphiuridae	1	0	0	0	1
Total echinoderms	24	20	11	2	57
Cnidaria					
Unidentified Actiniaria	3	5	4	3	15
Rhynchocoela					
Zygonemertes virescens	0	1	0	0	1
All phyla					
Individuals	313	335	168	121	937
Species	20	25	16	14	31

[a] Replicates were pooled.

TABLE 5

AVERAGE NUMBER OF ANIMALS AND AVERAGE NUMBER OF SPECIES IN CONTROL AQUARIA AND IN AQUARIA EXPOSED TO TCB VIA SEDIMENT

Taxon	Control	TCB (μg/g), nominal		
		10	100	1000
Arthropoda	23.7 (4.3)[a]	18.0 (5 0)	12.7 (3.5)	6.8* (1.3)
Annelida	10.0 (1.2)	11.2 (0.9)	7.7 (1.1)	6.0* (0.9)
Mollusca	12.5 (3.0)	15.5 (3.1)	3.5* (0.5)	0.7* (0.2)
Chordata	1.5 (1.1)	6.8 (4.3)	1.7 (1.3)	5.8 (2.9)
Echinodermata	4.0 (0.9)	3.3 (0.6)	1.8* (0.5)	0.3* (0.3)
Cnidaria	0.5 (0.3)	0.8 (0.2)	0.7 (0.3)	0.5 (0.3)
Rhynchocoela	0	0.2 (0.2)	0	0
All Phyla				
Individuals	52.2 (7.8)	55.8 (9.0)	28.0* (3.5)	20.2* (3.5)
Species	11.5 (0.8)	13.0 (0.8)	9.2* (0.5)	7.0* (0.7)

[a] Standard error in parentheses.
* Significantly different from control ($\alpha = 0.05$, analysis performed on square-root transformed counts).

was affected by sediment-bound TCB and probably also by waterborne TCB (counts less than control but too sparse to justify an ANOVA).

Concentrations of TCB that caused significant differences in abundance among benthic organisms were two orders of magnitude higher for sediment-bound exposure than for waterborne exposure. In nature, however, sedimentary sinks can contain many toxic substances at concentrations much higher than the concentrations in the overlying water (Schuytema *et al.*, 1984). Concentrations of pesticides as high as 6000 ppm (μg/g) have been reported for marine littoral zone sediments near a pesticide plant in Denmark (Mohlenberg and Kiorboe, 1983).

TCB in interstitial water or that leached into the water column from contaminated sediments may have contributed to mortality in communities exposed via the sediment. However, the source of the chemical was the sediment. Concentrations of the toxicant in samples of water overlying sediments containing 100 or 1000 μg/g occasionally exceeded the lowest concentration (average, 0.04 mg/liter) that was toxic to communities exposed via water.

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